Level Vitamin D, Calcium Serum and Mandibular Bone Density in HIV/AIDS Children

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Abstract

Human immunodeficiency virus (HIV) is a virus attacking the immune system of the body, usually was caused by HIV type 1. The proportion of women in new HIV infections in Indonesia has grown from 34 percent in 2008 to 44 percent in 2011 will lead a rise infections among children. There is an association between low vitamin D and HIV disease progression. Vitamin D is not only involved in calcium homeostasis which has a negative impact on bone health, but also in the regulation of the immune system. Bone alteration has been observed in the course of HIV which reduced bone mineral density is the bone alteration found in HIV patients. Bone mineral density is a parameter that predicts fracture risk which in turn correlates with a shorter life expectancy. This research will study the level vitamin D and calcium serum with mandibular bone density in HIV/AIDS children. The research method is cross-sectional study, serum 1,25-dihydroxyvitamin D and calcium levels were assessed from blood for the randomly selected subject of HIV-infected children enrolled treatment at Clinic Teratai FKUP Rumah Sakit Hasan Sadikin Bandung, West Java, Indonesia during March-June 2015. A panoramic radiograph was taken for measuring mandibular bone density. All 40 subject HIV/AIDS children showed serum 1,25-dihydroxyvitamin D were classified as vitamin D deficient (≤ 20nm/ml). A few subject showed an insufficient serum calcium level and 70%patient has low mandibular bone density. Deficient vitamin D levels may lead lower mandibular bone density in HIV/AIDS children.

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Introduction

Human immunodeficiency virus (HIV) is a virus causing *acquired immunodeficiency syndrome* (AIDS). Researchers conducted in several countries showed a rapid increase in infected women, which correspondingly increase the number of HIV-infected children. HIV infection is passed from infected mothers to their children through vertical transmission: through the placenta during pregnancy (intrauterine), at birth (intrapartum), and after birth through breastfeeding. To date, it has been reported that as many as 3.3 million children were infected with HIV.[1] The proportion of women in new HIV infections in Indonesia has grown from 34 percent in 2008 to 44 percent in 2011 which lead to an increasing number of infections among children.

Ever since the introduction of highly active *antiretroviral* therapy (HAART) in the mid-1990s, the incidence and mortality rate related to opportunistic infections and malignancies that occurred due to the low immune system has been dramatically decreased. In the other hand, the use of HAART caused various side effects, one of which is, according to several studies, the relationship between antiretroviral therapy (ART) and vitamin D deficiency and low bone density in infected patients.[2,3,4,5]

Numerous studies assessing bone mineralization and metabolism in adult patients receiving ART showed contradictory results.[6,7] Other studies involving HIV-positive children and teenagers receiving ART showed astonishing bone metabolism rate, which was assessed by

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bone turnover biochemical.[8,9,10] A longitudinal study also confirmed low bone mineral density, as well as bone metabolism abnormality in HIV infected children, treated with HAART.[11] Results of studies above showed that HAART was suspected to contribute to the decrease in bone mass and bone metabolism alteration in HIV-infected children.

Klinik Rawat Jalan Teratai FKUP - RS. Dr. Hasan Sadikin Bandungis an outpatient clinic serving as the center of assistance and research for patients with HIV in West Java Province, Indonesia. The total number of children patients with HIV in Klinik Teratai was 170 children. This study was aimed at obtaining data of calcium serum level, vitamin D, level, and mandibular bone density in children with HIV receiving HAART therapy for more than 3 years.

Materials and methods

This is a cross-sectional study with a population of HIV-AIDS infected children in Klinik Rawat Jalan Teratai FKUP- RS. Dr. Hasan Sadikin Bandung aged below 15 years old. The sampling method used was consecutive sampling method, which means that all samples meeting the inclusion criteria were included in this study.

The inclusive criteria were: 1) HIV- AIDS patients receiving at least 3 years of HAART treatments in Klinik Teratai 2) patients without genetic abnormalities 3) patients without other growth and developmental abnormalities.

The study was conducted after obtaining Health Research Ethics and parental permission by signing informed consent. Blood from children meeting the inclusion criteria was taken in order to examine its calcium serum and vitamin D level.

As much as 200 µl of blood was needed in order to test the calcium serum level using *O-cresol Phthalein*method, meanwhile, 300 µl of blood was necessary for testing the vitamin D level using the same method. Mandibular bone density was measured using panoramic radiograph. The measurement was conducted using 1 set of computer and line strength method in order to measure bone quality: bone density was measured from the trabeculae of mandibular cortex as radioopaque (white) area in the radiograph; Region of Interest (ROI) area was defined as a square area, used for measuring pixel; Pixel was a representation of the smallest dot in a graphic picture with unit of Dot Per Inch. Dot Per Inch was a group of dots in 1- inch linear form (1 inch= 2.54cms). A 30x30 pixels ROI were established in left and right posterior of the mandibles below the alveolar bone.

Processing radiograph for microstructure analysis of bone trabeculae using Adobe Photoshop software. Cropped images were filtered using high pass filtering technique with the use of Gaussian blur in order for the brightness variation caused by different object widths and soft tissue superimpositions to come out.

It was then followed by removing any fine and medium scale with high-density scale. Binarization in the images with brightness by showing the trabeculae and bone marrow. Binarization images were erased three times and dilated to remove noise. The bone trabeculae area were then measured.

Results

As many as 40 children from 6 to 15 years of age with HIV/AIDS receiving HAART treatment for at least 2 years who met the inclusive and exclusive criteria were included in this study at Klinik Teratai FKUP RSHS Bandung. Samples taken were blood for examining the calcium serum and vitamin D level as well as panoramic radiograph to obtain mandibular bone density.

Results showed the mean of calcium serum level in children with HIV was 9.32± 0.09mg/dl. The mean of vitamin D level in children with HIV was 18.84±1.2 mg/ml. Mandibular bone density was conducted in the trabeculae and mandibular cortical bone.

The examination on the bone trabeculae area was featured in a fractal dimension that showed the mean density in children with HIV was 22.8±2.9.

The examination of the cortical bone area was featured in mandibular cortical index and categorized into: 1) c1 showed normal cortical bone, which was defined as distinctive and soft endosteal margin (Figure 1); 2) c2 showed osteopenia of cortical bone which was defined by erosion in endosteal margin (Figure 2); 3) c3 showed osteoporosis of cortical bone which was defined by severe erosion and in distinctive margin (Figure 3).

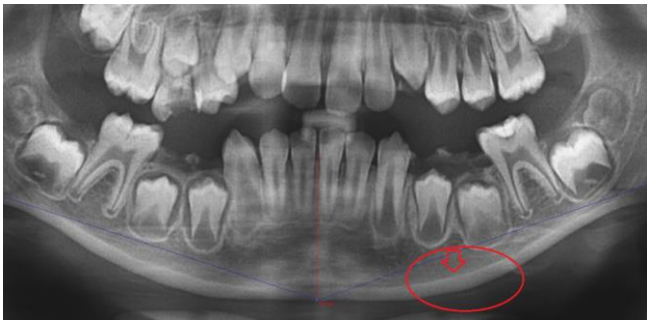


Figure 1. Normal mandibular cortical index.

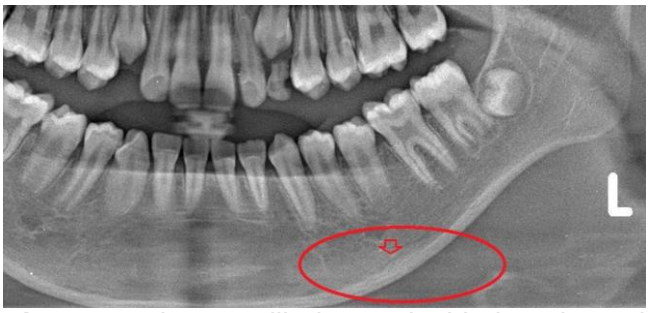


Figure 2. The mandibular cortical index showed osteopenia.

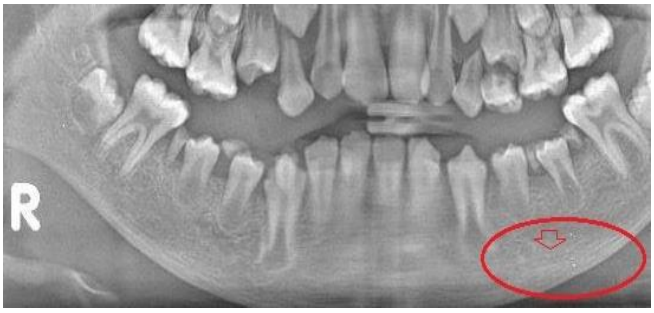
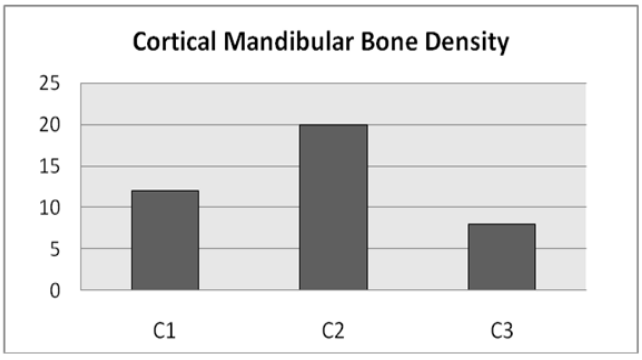


Figure 3. The mandibular cortical index showed osteoporosis.



Graphic 1. Cortical Mandibular Bone Density, C1 (normal bone density); C2 (osteopenia); C3 (osteoporosis)

The study showed that mandibular cortical index in HIV children were as followed: C1 30%, C2 50% and C3 20% (Graphic 1).

Discussion

HIV virus infected human T cells causing immune dysfunction. Patients suffering from AIDS showed the decrease of CD4 reaching less than 200 cell/mm^3 or patients experienced opportunistic infection, such as candidiasis or pneumonia. HIV/ AIDS patients' life expectancy increase as development in diagnostics and therapy increase, however, chronic complication were also potentially developed. The result of this study showed vitamin D metabolism disorder in HIV patients, especially those receiving HAART therapy.

The mean of calcium serum level in children with HIV was 9.32±0.09 mg/dl, which showed within normal limits of calcium level. Calcium is important as an intracellular and extracellular cation in the physiological process, such as in muscular contraction, vascular, and hormone secretion. Calcium level in the human body is always maintained in the balance between calcium absorption, bone remodeling, and calcium excretion. Several studies showed that in low calcium intake, human body had adaptation mechanism to maintain blood calcium level, called calcium homeostasis. The previous theory explained that if low calcium level in blood occurred, calcium would be released from bone under control of vitamin D and parathyroid hormone (PTH). Garg et al in 2014 introduced local adaptation mechanism in the digestive system, called intestinal calcite, that maintain calcium level homeostasis in blood. Intestinal calcite was defined as calcium in digestive system were absorbed optimally by the body and regulated the active form of vitamin D, influencing calcium absorption. Calcium homeostasis was maintained even though vitamin D insufficiency occurred.[12]

The mean of vitamin D level in children with HIV was 18.84±1.2 ng/ml, showing vitamin D deficiency in children with HIV. Vitamin D deficiency was defined as the serum level of 1,25 hydroxy vitamin D (25OHD) was lower than 20 ng/ml. Several previous studies showed a decrease in vitamin D level among HIV/ AIDS patients, in relation to the severity. A cohort study conducted by Rustein et al showed that children infected perinatally experienced vitamin D deficiency compared to healthy children as a control.[13] Wiboonchutikul et al stated that prevalence of vitamin D deficiency in HIV

patients were high although patients lived in the tropical area; and that there was no difference in vitamin D level in patients receiving ART therapy and patients without ART therapy.[14] However, the study concealed that efavirenz medicament was significantly related to vitamin D deficiency status in HIV patients. The effect of efavirenz on vitamin D metabolism is hypothesized to occur through the induction of 24-hydroxylase, a cytochrome P450 enzyme, that inactivates 25OHD and 1,25OHD.[15]

The decrease mechanism of vitamin D level in HIV/ AIDS patients occurs when HIV virus increased cytokine TNFα due to inflammation and affected hydroxylation in kidneys. The increase of macro flag and lymphocytes consumption of 25(OH)D was in line with the degree of severity. Decreases the level of vitamin D depended on antiretroviral medicament used, such as those belonging to protease inhibitor group that inhibited hidroksilasi 25(OH)D. Meanwhile, the nonnucleoside reverse transcriptase inhibitor increased the catabolism process of 25(OH)D and 1,25$(OH)_2$D.[16] Campbell showed that vitamin D deficiency mechanism affected natural immunity against HIV infection through TLR 8 agonist stimulation that improves YP27B1 and VDR expression regulation inducting CAMP and autophagic process. It supported vitamin D advantages and important roles in controlling HIV infection.[17]

The result of mandibular bone density through panoramic radiograph showed mandibular cortical index in HIV children were as follows: C1 30%, C2 50%, and C3 20%. Vitamin D deficiency was related to the progressivity of disease severity in HIV-infected patients resulted in a great tendency of reduced bone density and osteoporosis. Vitamin D mechanism affected the bone mass through bone remodeling causing weight loss and function capacity disorder related to OPG/KANKL/RANK system. Bone remodeling is a complex process involving hormones that also play role in calcium homeostasis (PTH, calcitriol, calcitonin, estrogen, and androgen). Hormone interacted with local factors, such as IL1, IL-6, TGF, TNF, CSF; which all involved in OPG/RANKL/RANK system as the final process of osteoclast genesis.[17,18,19]

Another study showed that HIV-infected patients with very low 1,25(OH)2D3 level had PTH production stopped although calcitonin level in their blood was normal. PTH increased calcium absorption triggering RANKL expression in osteoblasts, therefore, stimulated osteoclast maturation. Teichman conducted a study on osteopenia occurrence in women HIV patients accepting HAART therapy, especially those in protease inhibitor group, which showed a relation between the low bone formation marker and bone reabsorption marked by increased calcium excretion. Furthermore, the decreased level of 1,25(OH)2D3 contributed towards calcium level imbalance and bone formation inhibition.[20,21]

HIV infection also decreased monosit, macrophage, and TNF receptor counts, causing the low PTH receptor and cAMP respond playing role in PTH stimulation production.[18,22,23] Vitamin D mechanism causing osteopenia and osteoporosis was still unknown, however, in HIV patients, it was related to the length of infection, high viral load, lactate level, and alkaline phosphatase. Therefore, adequate vitamin D level and PTH level has to be evaluated as early as possible in HIV-infected patients. Several studies explained above showed direct relationships between HIV infection and vitamin D deficiency. Effect of interactions of 1,25(OH)2D3 was modulated with vitamin D receptor (VDR), therefore, it facilitated the bonding between the nucleus and vitamin D receptor element (VDRE) which helped gene transcription.[20,21] VDR gene expression occurred not only on skeletal tissue system but also in monocyte, macrophage, dendritic cells, natural killer cells, T cells as well as B cells facilitating vitamin D immunomodulatory effect.[18,23,24]

Conclusions

Vitamin D immunomodulatory effect was related to VDR gene polymorphism and various HIV/ AIDS degrees of severity which may lead to lower mandibular bone density in HIV/AIDS children.

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Declaration of Interest

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